THE RESPONSE OF FOUR SOIL FUNGI TO PARAQUAT TREATMENT

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SUMMARY. – The response of 4 soil fungi in culture to paraquat treatment was assessed using various concentrations of paraquat in PDA. The linear growth and sporulation rates and changes in colony morphology were monitored. The growth rates of *Gliocladium virens*, *Trichoderma hamatum* and *T. koningii* were less susceptible to paraquat than *Humicola fuscoatra* which was slightly inhibited at the recommended field concentration, and totally inhibited at 1.2×10^5 mg/l. Sporulation in *T. hamatum* and *T. koningii* was decreased at the recommended field concentration while that of *G. virens* was stimulated. The treated fungi recovered their growth and sporulation when transfered to paraquat-free PDA. Changes in colony morphology included reduced pigmentation and enhanced chlamydospore formation.

RÉSUMÉ. – La réponse des cultures de quatre champignons du sol à un traitement au paraquat est examinée à diverses concentrations dans le P.D.A. La croissance linéaire, le taux de sporulation et les transformations morphologiques de la colonie sont mesurés. Les croissances de *Gliocladium virens*, *Trichoderma hamatum* et *T. koningii* sont moins sensibles au paraquat que *Humicola fuscoatra* qui est légèrement inhibé aux concentrations conseillées aux champs, et totalement arrêté à 1.2×10^5 mg/l. La sporulation de *T. hamatum* et *T. koningii* est réduite aux concentrations utilisées en agriculture, tandis que celle de *G. virens* est stimulée. Les champignons traités récupèrent leur croissance et leur sporulation normales après transfert sur P.D.A. sans paraquat. Les changements morphologiques des colonies concernent une réduction de pigmentation et la formation de chlamydospores.

KEY WORDS : Soil fungi, herbicide, paraquat, *Gliocladium virens*, *Trichoderma hamatum*, *Trichoderma koningii*, *Humicola fuscoatra*.

INTRODUCTION

The application of herbicides to control weed growth in agricultural soils, green houses, orchards and even parks has been on the increase. The use of such chemicals is beneficial, but residual herbicides can also affect non-target organisms such as soil fungi. Such effects can lead to changes in activities of species

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involved in nutrient recycling as well as those which are phytopathogenic. Various workers have demonstrated that herbicides may be stimulatory, inhibitory or toxic to specific groups of soil fungi (WILKINSON & LUCAS, 1969; JONES & WILLIAMS, 1971; ANDERSON & DREW, 1976; FILHO & DHINGRA, 1980; CERKAUSKAS & SINCLAIR, 1982; EL-ABYAD & al., 1983). Paraquat is one of the common herbicides used in the control of weeds in Singapore. This report presents data from an assessment of the effects of this herbicide on the growth, sporulation and colony characteristics of 4 common soil fungi.

MATERIALS AND METHODS

– **Test herbicide** : a commercial formulation of paraquat (1,1'dimethyl-4, 4' bipridyliumion) was used. Filter-sterilized stock herbicide was incorporated into 39 g/l sterile potato dextrose agar (PDA, Difco) to give a final range of herbicide concentration in the agar medium. This range consisted of the recommended field concentration (1.2×10^3 mg/l) as well as 10x below, and 10x and 100x above the field concentration. The paraquat-PDA was dispensed 20 ml per petri-dish. Control plates consisted of PDA without the addition of paraquat.

– **Test fungi** : four common fungi namely *Gliocladium virens* Miller, Giddens & Foster, *Trichoderma hamatum* (Bon.) Banier, *Trichoderma koningii* Oudemans and *Humicola fuscoatra* Traaen were tested. Four to seven days old pure cultures of each fungus were first grown on PDA plates. Mycelial discs were then cut out using a sterile steel borer of 1 cm diameter. A disc each was aseptically transfered to the centre of the control and paraquat-PDA plates. Three replicates plates were prepared for the control and each herbicide concentration. The plates were incubated at 28°C and the following daily observations were made :

* Linear growth rate : two measurements (mm) of the fungal colony diameter, one at right angles to the other, were taken until growth had reached the edge of the plate. The average of these 2 measurements divided by the time taken gave the linear growth rate of the colony in mm / day.

* Sporulation rate : two streaks across the diameter of the colony, one at right angles to the other, were made with an inoculation needle. The spores collected were dispersed in 10 ml of distilled water in a test tube. The average number of spores per ml of the spore suspension per day was counted using a Neubauer-Improved haemacytometer.

* Colony morphology : colony morphology such as colony shape, texture and colour of paraquat-treated cultures were compared to the control cultures. In addition, small amounts of fungal materials were mounted in lactophenol cotton blue and examined under the light microscope.

* Recovery test : the ability of the paraquat-treated cultures to grow on paraquat-free PDA was assessed by transferring mycelial discs from cultures treated with the highest test paraquat concentration to PDA plates. The linear growth rate,

sporulation rate and colony morphology were observed as before. Recovery in growth and sporulation was expressed as a percentage of the respective rates of the controls.

RESULTS AND DISCUSSION

The results showing the effect of paraquat on the linear growth rate of *G. virens*, *T. hamatum*, *T. koningii* and *H. fuscoatra* are shown in Figure 1a. The growth rate of *G. virens*, *T. hamatum* and *T. koningii* did not differ much from that of the controls (24.0 - 26.0 mm/day) when treated with up to 10 x the recommended field concentration of paraquat. However, at the higher concentration of 1.2×10^5 mg/l, the growth of all 3 fungi declined sharply to between 4.5 mm-11.0 mm/day. *H. fuscoatra* was a slow grower, as shown by the growth rate of the control (3.5 mm/day). This was maintained in the presence of up to 1.2×10^2 mg/l paraquat. At higher concentrations, growth gradually declined, and was totally inhibited by 1.2×10^5 mg/l of the herbicide.

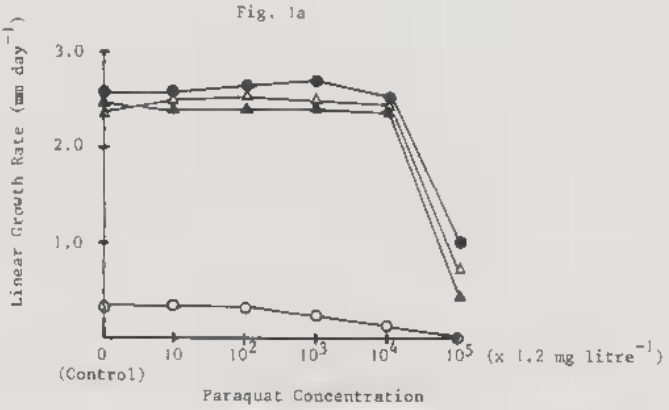

Figure 1a. — Effect of paraquat on growth of test fungi.
△—△ *Gliocladium virens*; ●— —● *Trichoderma hamatum*; ▲—▲ *Trichoderma koningii*; ○—○ *Humicola fuscoatra*.

Figure 1a. — Effet du paraquat sur la croissance des champignons.

The inhibitory effects of paraquat on the growth rate of soil fungi have also been observed by other workers. However, the inhibitory concentrations reported varied from fungus to fungus. WILKINSON & LUCAS (1969) observed that *Eurotium* spp., *Fusarium culmorum*, *Helminthosporium sativum*, *Ophiobolus graminis* and *Trichoderma viride* were tolerant to paraquat concentrations of up to 100 ppm, but were severely inhibited at 500 ppm and 1000 ppm. A much lower inhibitory concentration of 25 ppm for some other fungi were documen-

ted by SZEGI (1970). A comparison within this study shows that *G. virens*, *T. hamatum* and *T. koningii* were less susceptible than *H. fuscoatra* which was slightly inhibited at the recommended field concentration and totally inhibited at 1.2×10^5 mg/l of the herbicide.

SMITH & LYON (1976) suggested that the susceptibility of fungi to paraquat is strongly correlated with the accumulation of the herbicide in the mycelium. This may account for the ability of the test fungi to recover their growth upon their transfer onto herbicide-free PDA (Figure 1b). However, recovery was only 76 % and 74 % for *T. koningii* and *T. hamatum* respectively, and 66 % for *G. virens*.

Fig. 1b

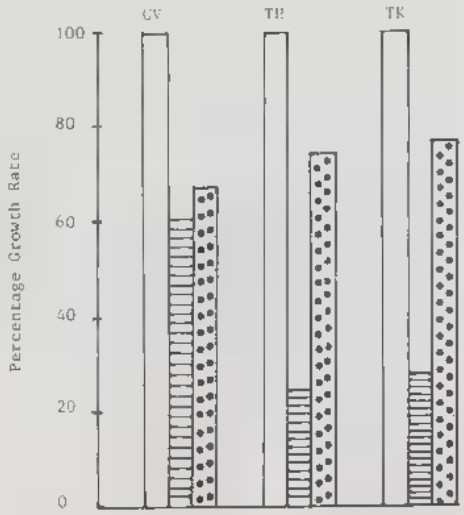

Figure 1b. – Recovery of growth from paraquat treatment.

GV : *Gliocladium virens*; TH : *Trichoderma hamatum*; TK : *Trichoderma koningii*.
☐ Control (PDA without paraquat); ▤ Treated (PDA with 1.2×10^5 mg/l paraquat);
▣ Recovery (PDA without paraquat).

Figure 1b. – Rétablissement de la croissance après traitement au paraquat.

The sporulation of the test fungi responded variously to paraquat treatment (Figure 2a). *H. fuscoatra* sporulated poorly in both the control and treated cultures. A gradual increase in sporulation with herbicide concentrations of up to 1.2×10^4 mg/l was observed for *G. virens*. At 1.2×10^5 mg/l of the herbicide, sporulation rate fell to half of the control (0.22×10^6 spores/ml/day). For *T. koningii*, sporulation generally declined with increasing herbicide concentrations.

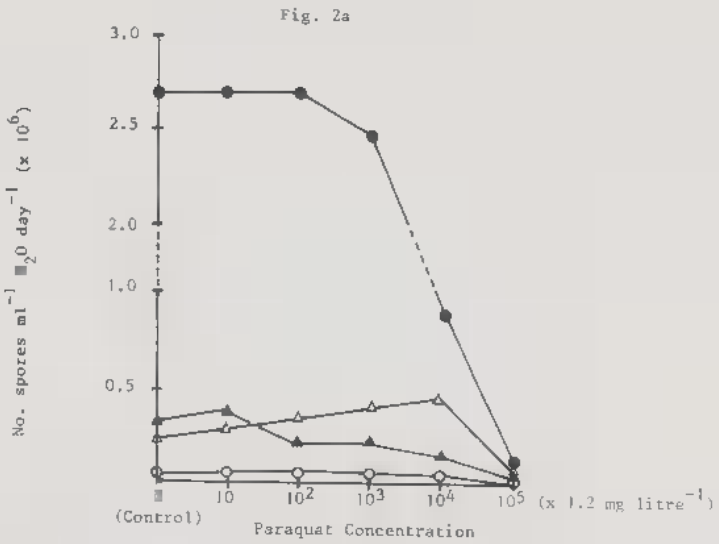

Figure 2a. — Effect of paraquat on sporulation of test fungi.
△—△ *Gliocladium virens*; ●- -● *Trichoderma hamatum*; ▲—▲ *Trichoderma koningii*; ○—○ *Humicola fuscoatra*.

Figure 2a. — Effet du paraquat sur la sporulation des champignons testés.

This decline was very marked for *T. hamatum*. The rate of 2.75 x 10^6 spores/ml/day for the control fell to 0.1 x 10^6 spores/ml/day in the presence of 1.2 x 10^5 mg/l paraquat. Again, the effect of paraquat on fungal sporulation can be inhibitory or stimulatory, depending on the herbicide concentration and fungus used. Inhibition of sporulation has been reported for *Septoria nodorum* and *S. tritici* (JONES & WILLIAMS, 1971) and enhancement for *Rhynchosporium secalis* (STEDMAN, 1977). In this study, *G. virens* was more tolerant in conidiation to the recommended field concentration, compared to *T. hamatum* and *T. koningii*. This was also reflected in the percentage recovery in sporulation (Figure 2b). *G. virens* recovered to 77 %, *T. koningii* 28.5 % and *T. hamatum*, a low 16 %.

Changes in fungal colony morphology such as reduced pigmentation and formation of irregular colony margins in response to herbicide treatments have been reported by WILKINSON & LUCAS (1969). Such changes were evident in *G. virens*, *T. hamatum* and *T. koningii*. Cultures of the 3 fungi formed more sterile aerial hyphae and appeared less green in pigmentation in response to 1.2 x 10^5 mg/l paraquat. In addition, all 3 fungi produced abundant chlamydospores under treatment.

Morphological changes in *H. fuscoatra* were observed at a lower paraquat concentration of 1.2 x 10^4 mg/l. The colonies showed lesser brown pigmentation and «feathery» colony margins. The hyphae also appeared «beaded» and

Fig. 2b

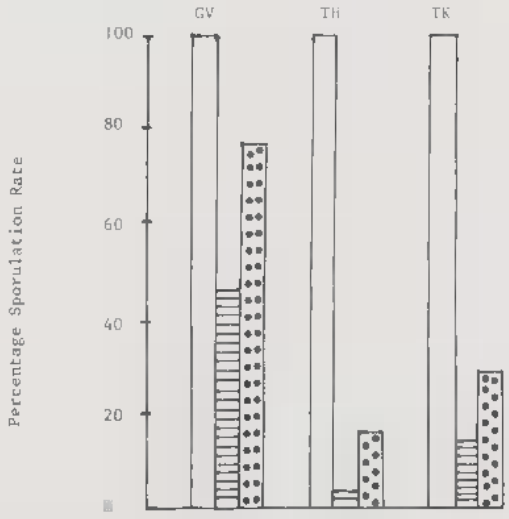

Figure 2b — Recovery of sporulation from paraquat treatment.
GV : *Gliocladium virens;* TH : *Trichoderma hamatum;* TK : *Trichoderma koningii.*
☐ Control (PDA without paraquat); ▤ Treated (PDA with 1.2 x 10^5 mg/l paraquat);
▦ Recovery (PDA without paraquat).

Figure 2b. — Rétablissement de la sporulation après traitement au paraquat.

formed abundant large chlamydospores. The observed reduction in pigmentation of treated cultures could be attributed to reduced sporulation and enhanced formation of sterile hyphae.

The increased formation of chlamydospores by all the test fungi could have contributed to the ability of the fungi to recover their growth on herbicide-free PDA, since these resistant spores can germinate and produce mycelial growth under favourable conditions of growth.

REFERENCES

ANDERSON J.R. and DREW E.A., 1976 — Effect of pure paraquat dichloride, «Gramoxone W», and formulation additives on soil microbiological activities. I. Estimations of soil biomass in laboratory-treated soil. *Zentralbl. Bakteriol. Hyg., 2. Abt.* 131 : 125-135.

CERKAUSKAS R.F. and SINCLAIR J.B., 1982 — Effect of paraquat on soybean pathogens and tissues. *Trans. Brit. Mycol. Soc.* 78 : 495-502.

EL-ABYAD M.S., ISMAIL I.K. and AL-MESHHADANI S.A., 1983 — Effects of some biocides on *Fusarium oxysporum* formae speciales causing cotton and tomato wilts

in Egypt. *Trans. Brit. Mycol. Soc.* 80 : 283-287.

FILHO E.S. and DHINGRA O.D., 1980 — Effect of herbicides on survival of *Macrophomina phaseolina* in soil. *Trans. Brit. Mycol. Soc.* 74 : 61-64.

JONES D.G. and WILLIAMS J.R., 1971 — Effect of paraquat on growth and sporulation of *Septoria nodorum* and *Septoria tritici*. *Trans. Brit. Mycol. Soc.* 57 : 351-357.

SMITH S.N. and LYON A.J.E., 1976 — The uptake of paraquat by soil fungi. *New Phytol.* 76 : 479-484.

STEDMAN O.J., 1977 — Effect of paraquat on the number of spores of *Rhynchosporium secalis* on barley stubble and volunteers. *Pl. Pathol.* 26 : 112-120.

SZEGI J., 1970 — Effect of some herbicides on the growth of cellulose decomposing microscopic fungi. *Meded. Fac. Landbouwwetensch. Rijksuniv.* 35 : 559-561.

WILKINSON V. and LUCAS R.L., 1969 — Effects of herbicides on the growth of soil fungi. *New Phytol.* 68 : 709-719.